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The Spectrum of Free Neuraminic Acid Storage Disease in Childhood: Clinical, Morphological and Biochemical Observations in Three Non-Finnish Patients

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N-acetylneuraminic acid (sialic acid) storage disease is a rare autosomal recessive lysosomal disorder. Clinically two major forms exist, an infantile type with severe progression leading to early death, and a milder form (Salla disease) with a protracted course. Intermediate forms may also exist. Diagnosis rests on the determination of an excessive excretion of sialic acid in urine and concomitant storage in fibroblasts, the severe forms exhibiting the highest excretion and storage. We present clinical, morphological, and biochemical data on three non-Finnish patients with sialic acid storage disease. Patient 1 was a preterm infant with neonatal ascites, coarse face, hepatosplenomegaly, pale skin, and wispy hair. Vacuolated lymphocytes were abundant in a peripheral blood smear and he excreted large amounts of free sialic acid. High levels of free sialic acid were also found in cultured skin fibroblasts. He died at age 6 months from progressive respiratory insufficiency. Patient 2 was an 11-month-old Egyptian girl with coarse face, frequent upper respiratory tract infections, hepatosplenomegaly, and severe psychomotor retardation. Sialic acid excretion was elevated, likewise the storage in fibroblasts. Histological investigations documented vacuolar storage in a skin biopsy and in iliac crest tissue. Patient 3 was a 16-year-old girl with slightly coarse face, severe generalized muscular hypotonia, ataxia, and kyphoscoliosis originally diagnosed as having postpartum asphyxia. She suffered progressive motor function loss and had dysarthria. Urinary sialic acid was elevated and a skin biopsy demonstrated vacuolization. The clinical variability of sialic acid storage disease is exemplified by these three cases. Simple urinary screening for free sialic acid facilitates the diagnosis. The degree of urinary excretion may indeed correlate with clinical presentation and progression.

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KEY WORDS: sialic acid, lysosomal disease, Salla disease, variability

INTRODUCTION

The first patients with severe psychomotor retardation, ataxia and lysosomal storage of free sialic acid were reported by Aula et al. [1979]. The disorder, termed Salla disease (SD) after the birthplace of the patients, has been considered to be a uniquely Finnish entity with ca. 90 patients known [Gahl et al., 1995], although sporadic patients of other ethnic backgrounds have been described [Wohlburg-Buchholz et al., 1985; Echenne et al., 1986; Ylitalo et al., 1986 ; Baumkötter et al., 1986; Mancini et al., 1992]. Symptoms usually appear between age 6 and 9 months starting with hypotonia and ataxia [Renlund et al., 1983]. Horizontal nystagmus may be present even earlier but disappears gradually. Ataxia may be truncal or distal and may be accompanied by athetosis. After age 1 year, many patients develop spasticity and 25% never learn to walk [Mancini et al.,1991]. Skeletal abnormalities and organomegaly are not present. Adults are severely mentally retarded and most patients utter only single words or short sentences [Gahl et al., 1995]. Absence-like epilepsy is com-

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Abbreviations: SD = Salla disease; ISSD = Infantile sialic acid storage disease

Dedicated to Jürgen W. Spranger on the occasion of his 65th birthday with admiration and best wishes.

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mon, but generalised tonic-clonic seizures are infrequent and occur only in severely affected patients. The life expectancy is considered normal.

In 1982 a more severe infantile phenotype of sialic acid storage disease (ISSD) was described [Hancock et al., 1982 ; Tondeur et al., 1982]. These patients have failure to thrive, visceral involvement, generalized edema, and a very poor prognosis. At present only 15 cases have been described worldwide [Gahl et al., 1995].

Both SD and ISSD have the same biochemical abnormality, namely, a defective lysosomal membrane transport system for sialic acid [Renlund et al., 1986 ; Tietze et al., 1989] despite distinctly different clinical phenotypes. The intra-lysosomal storage and increased urinary excretion of sialic acid are the hallmark of this disorder. Patients with ISSD excrete distinctly more sialic acid than patients with SD [Gahl et al., 1995], a fact which may correlate with clinical severity.

We present clinical, histological, electron microscopic, and biochemical observations in three patients with sialic acid storage disease, illustrating the phenotypic variation, simplicity of diagnosis, and providing new evidence of storage in cartilaginous tissue.

PATIENTS AND METHODS

Patient 1

This child, the first of non-consanguineous German parents, was born prematurely in the 29th week of gestation (birth weight 2,200 g). Ascites, which had been detected in the 13th week, had increased steadily. An amniocentesis and chromosome analysis gave normal results. Further intrauterine development was normal. At birth, the infant had an enlarged abdomen, large bilateral inguinal hernias, edema, and somewhat coarse facial appearance with a long philtrum and sparse eyebrows (Fig. 1). The baby was initially intubated and a laparotomy gave no indication of the cause of the ascites except that the liver was swollen and fragile. After 5 days, the patient was extubated but up to the 3rd month of life had a high oxygen demand. Ultrasound examination of the kidneys and liver demonstrated an increased echogenicity. The heart was structurally normal but had reduced contractility which necessitated digoxin treatment. Liver and renal function was normal.

A peripheral blood smear showed a large number of vacuolated lymphocytes. Qualitative urinary screening [Sewell, 1988] demonstrated an increased excretion of free sialic acid. Quantitative analysis [Warren et al., 1959] gave a urinary excretion of free sialic acid of 1,069 μmol/mmol creatinine (normal = <300), thus suggesting the diagnosis of ISSD. Subsequent confirmation in cultured skin fibroblasts showed an increased amount of free sialic acid (66.33 nmol/mg protein; normal = 2.2–14.9) (Table I).

The patient was tube-fed and drank with difficulty but was discharged at age 2 months. Frequent respiratory infections, twice with pneumonia, complicated the clinical picture. At age 5 months he became cyanotic with a limited ventricular function although ascites, hepatomegaly, and splenomegaly had reduced. Renal echogenicity remained, although proteinuria could not be detected. Muscle tone became increasingly poorer with lack of head control. At the age of 6 months, cyanotic episodes increased in frequency which led to dyspnoea and hypercapnia from which he did not survive.

Patient 2

This child, an 11-month-old girl, was the product of consanguineous (1st cousins) Egyptian parents. Pregnancy and birth were unremarkable (birth length 51 cm; birth weight 2,100 g). At 9 months she was considered to be retarded in her development. She did not fix, laugh, roll over, or react to visual stimuli. She had also suffered frequent episodes of bronchitis and gas-

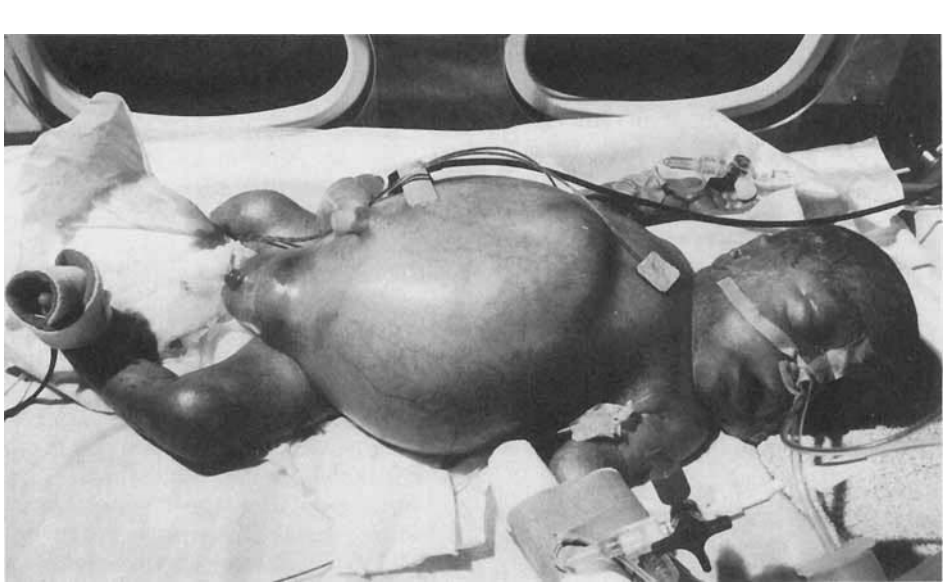

Fig. 1. Patient 1 postpartum exhibiting ascites and edema. (Reprinted from the Monatschrift für Kinderheilkunde, Springer-Verlag, with permission).

TABLE I. Urinary Excretion and Fibroblast Content of Free Sialic Acid in Patients

Patient	Urinary free sialic acid (μmol/mmol creatinine)	Fibroblast free sialic acid (nmol/mg protein)
1	1,060	66.33
Normal range[a]	<300	(2.2–14.9)
2	687	24.52
Normal range[a]	<150	(2.2–14.9)
3	122	n.d.
Normal range[a]	< 32	

[a]Age-related normal range. n.d. = not determined.

troenteritis. She presented at 11 months of age as a small (75 cm) severely motor- and mentally-retarded girl. She was microcephalic (OFC 42.5 cm, <3rd centile) with a somewhat depressed nasal bridge and a large tongue. Liver and spleen were enlarged (1.5 and 2.0 cm, respectively) and she had a small umbilical hernia. Reflexes were brisk. Rump and upper limbs showed muscular hypotonia. Routine blood chemistry values were normal. A radiological survey was unremarkable, bone age being consistent with that of an 11-month-old girl. Selective screening for disorders of complex carbohydrate metabolism demonstrated free sialic acid. Quantitation demonstrated an increased urinary excretion of free sialic acid (687 μmol/mmol creatinine; normal = <150), this being confirmed in cultivated skin fibroblasts (24.52 nmol/mg protein; normal = 2.2–14.9) (Table I). An iliac crest biopsy and a skin biopsy from this patient were fixed in 2.5% glutaraldehyde and 4% formaldehyde solutions. For histological studies, one iliac crest biopsy was decalcified in 5% formic acid. Both skin and iliac crest biopsies were embedded in paraffin and stained with hematoxylin and eosin, Giemsa, PAS-alcian blue, and toluidine blue. For electron micoscopy, the iliac crest biopsy was embedded without prior decalcification in low-viscosity epoxy resin using a modification of Schulz [1977]. Ultrathin sections were contrasted by uranyl acetate and lead citrate.

Histological examination of the skin biopsy demonstrated a regularly structured epidermis with plentiful fibres in the corium. Melanocytes and epithelial cells with vacuolated cytoplasm were seen in the stratum basale and stratum spinosum. Fibroblasts in the corium were somewhat enlarged and possessed small cytoplasmic vacuoles. Alterations were also seen in melanosomes suggesting degeneration of melanocytes as a possible consequence of vacuolar storage. Electron microscopic examination demonstrated fibroblasts and epidermal epithelial cells with multiple intracytoplasmic vacuoles (Fig. 2).

The histology of the iliac crest biopsy showed a moderately cell-enriched cartilaginous tissue with enlarged vacuolated chondrocytes. The cytoplasm reacted stronger with Alcian blue whereas the cartilage ground substance, in particular in the area of the resting zone, was only partially stained. Osteoblasts, osteocytes and the different cells of the bone marrow also showed a strongly vacuolated cytoplasm. Under the electron microscope, many optically empty storage vacuoles were found in chondrocytes (Fig. 3a), osteoblasts (Fig. 3b), osteocytes, and in bone marrow cells (Fig. 3c). Regular and uniform collagen fibers were present in the cartilage matrix. Light microscopy of a liver biopsy showed broad, swollen hepatocytes containing many cytoplasmic vacuoles.

The patient has returned to Egypt and is lost to follow-up.

Patient 3

This girl, the sole child of non-consanguineous German parents, was born with a birth weight of 3,200 g and a length of 52 cm after an uneventful pregnancy. Head circumference was 34 cm and the Apgar scores were 9/10/10. Birth was protracted and for a few moments she was cyanotic but recovered quickly under oxygen. Further development was normal. At 7 months the parents noted that she had no inclination to sit and was "weaker than other children." She had an expressionless face (Fig. 4a) and tended to hypersalivate. She never had horizontal nystagmus. She never learnt to crawl but could move about on all fours. She could utter ca. 15–20 words but her speech was unintelligible. Growth was normal. She was diagnosed as having a cerebral movement disorder with ataxia and athetosis. Mental development continued downhill and when seen at the age of 16 years (Fig. 4c), she had lost her vocabulary, suffered many pulmonary infections, developed joint contractures, and had severe kyphoscoliosis.

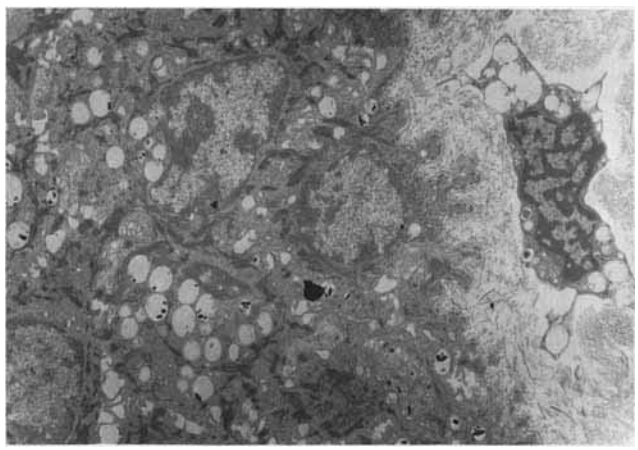

Fig. 2. Patient 2. Electron microscopy of skin biopsy showing lysosomal storage in epidermal epithelium and corium fibroblasts (x4,000).

Her neurological regression prompted urinary screening for metabolic diseases and free sialic acid was found. Quantitation resulted in an excretion of 122 μmol/mmol creatinine (normal = <32). Electron microscopic examination of a skin biopsy showed vacuoles, some membrane-bound, in mesenchymal and Schwann cells. Discrete vacuoles were also observed in sweat gland cells. The level of free sialic acid in this patient's cultured skin fibroblasts remains to be determined.

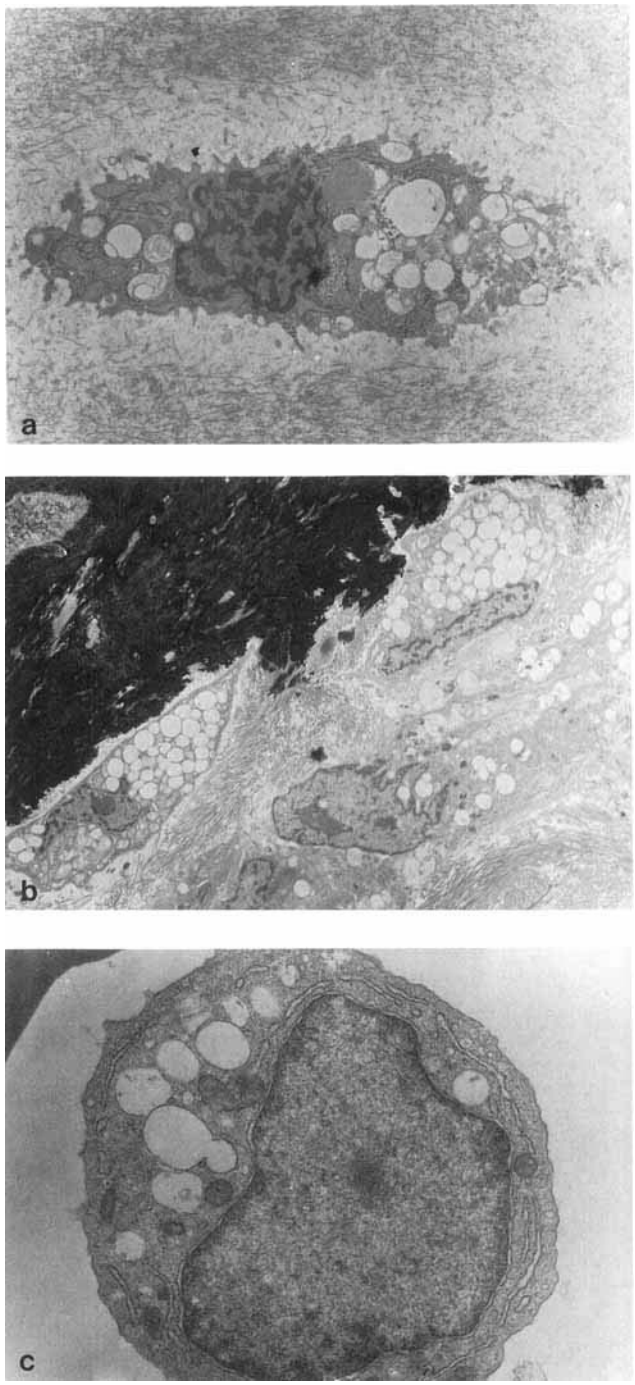

Fig. 3. **a:** Patient 2. Iliac crest cartilage chondrocyte with many cytoplasmic vacuoles (x4,500). **b**: Patient 2. Iliac crest osteoblasts with extensive cytoplasmic lysosomal storage lining the mineralisation zone (x2,000). **c**: Patient 2. Bone marrow cell (pronormoblast) with numerous lysosomal storage vacuoles in cytoplasm (x2,000).

DISCUSSION

These three patients exemplify the spectrum of sialic acid storage disease (Table II). These descriptions add to the variety of non-Finnish patients with sialic acid storage disease in that we have detected one case of ISSD and one case of SD in children of German parentage. Our Egyptian patient demonstrates further the panethnic distribution of sialic storage disease and the fact that these disorders may be much commoner in Egypt than in Western Europe [Temtamy et al., 1994]. Patient 1 fulfilled the criteria for ISSD. His primary symptoms consisted of severe statomotor and psychomotor retardation together with hypotonia of the lower limbs, hepatosplenomegaly, typical coarse face, pale blonde and wispy hair, and frequent upper respiratory tract infections [Bohnhorst et al., 1996]. Other symptoms included neonatal ascites [Gillan et al., 1984; Hancock et al., 1982], cardiomegaly [Cameron et al., 1990 ; Stevenson et al., 1983], umbilical and inguinal hernias [Stevenson et al., 1983 ; Tondeur et al., 1982] and foot deformities [Stevenson et al., 1983 ; Tondeur et al., 1982]. Other described symptoms of steroid-resistant nephrotic syndrome [Paschke et al., 1992; Sperl et al., 1990] and dysostosis multiplex [Stevenson et al., 1983] were not present. The cause of ascites in ISSD remains obscure.

Patients 2 and 3 can be classified as SD based on their protracted course. Patient 2 presented at a later date and did not show the severe clinical symptoms associated with ISSD. Both patients 2 and 3 had increased upper respiratory tract infections which are not usually present in SD patients [Gahl et al., 1995]. Patient 2 had somatic growth retardation whereas patient 3 was of normal height. Both patients had a progressive neurological degeneration and patient 3 had the typical loss of speech; in patient 2, the language barrier (German/Arabic) impeded a detailed investigation. Recently, a defective myelination pattern has been seen on MRI in some severely affected patients with SD [Haataja et al., 1994a]; unfortunately we were unable to undertake MRI in our patients to confirm this observation.

Morphological examination of skin from patients 2 and 3 demonstrated enhanced vacuolation, also present in the liver of patient 2. A previously undisclosed finding was the electron microscopic evidence of vacuolation in the iliac crest biopsy tissue of patient 2. These findings demonstrate the widespread lysosomal storage phenomena associated with SD and may be of use in helping to pinpoint the way to the diagnosis. Our patient with ISSD had marked vacuolation of peripheral lymphocytes. As of now, the effect of the increased storage of sialic acid on neuronal and brain metabolism remains obscure.

The differential diagnosis of ISSD and SD based on clinical grounds should not be difficult. Diagnosing SD in older patients may not be quite so easy. ISSD patients show a very high excretion of sialic acid (ca.

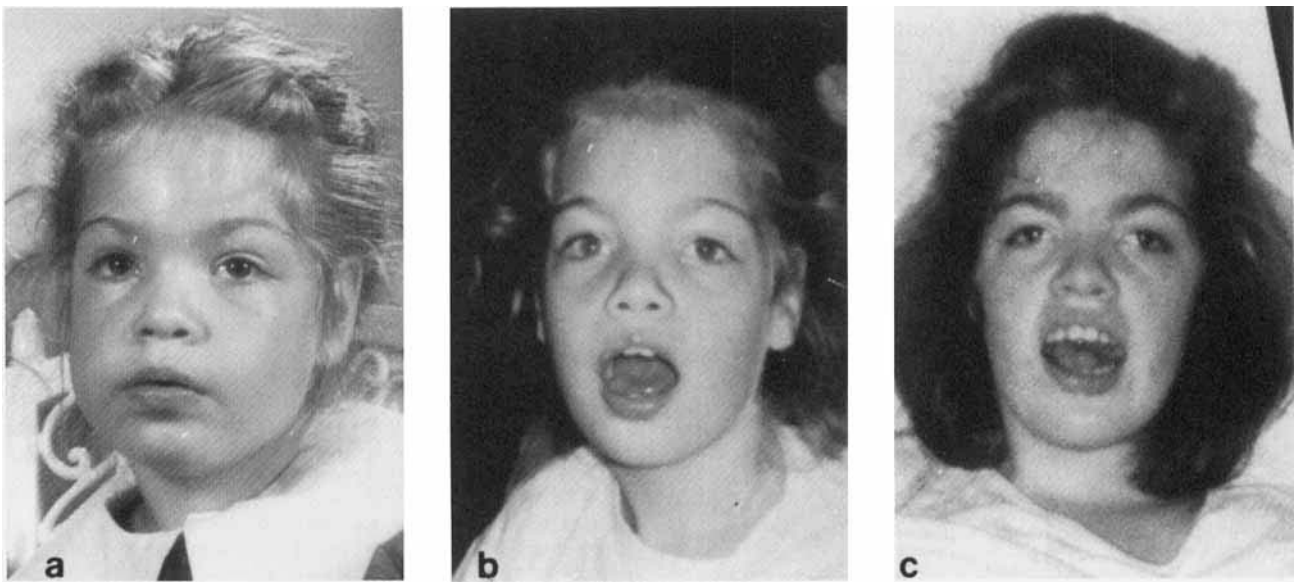

Fig. 4. Patient 3. Facial appearance at ages **a**) 6, **b**) 9, and **c**)16 years showing progressive coarsening.

1,100 μmol/mmol creatinine in our patient), whereas the excretion in SD patients can be variable. The urinary excretion of sialic acid in our patients is in line with that described for other patients [Gahl et al., 1995]. Nevertheless, a distinction should be made between ISSD and the extremely rare non-lysosomal disease sialuria [Fontaine et al., 1968; Wilcken et al., 1987] in which vast amounts of sialic acid appear in the urine, but the patients show no signs of vacuolar storage. Sialic acid is filtered but not reabsorbed by the human kidney and SD and ISSD patients have normal renal handling of sialic acid [Seppala et al., 1990].

Our patient 3 was diagnosed late (age 16 years) and then only when urinary screening was requested. Screening for free sialic acid by thin-layer chromatography should therefore be an integral part of a selective screening program. Quantitation of sialic acid can be accomplished by colorimetric or HPLC methods [Hommes, 1991], but since the excretion is age-dependent [Renlund, 1984], appropriate controls should be included. ^{1}H NMR analysis of native urine will easily demonstrate increased free sialic acid and appears to correlate well with colorimetric methods (Sewell, unpublished results). Confirmation of the diagnosis rests on the demonstration of increased free sialic acid in cultured skin fibroblasts. The levels in tissues reflect the urinary excretion and also the severity of the disease.

At present there is only palliative therapy for ISSD and SD. Prenatal diagnosis based on the demonstration of increased free sialic acid in chorionic villus tissue [Vamos et al., 1986 ; Lake et al., 1989] and amniotic fluid have been reported. Recent assignment of the genetic locus of SD to the long arm of chromosome 6 [Haatja et al., 1994b] in Finnish patients should enable prenatal diagnosis using linked markers at 6q with the promise of carrier detection in families of affected individuals. Further studies should be undertaken in patients of non-Finnish origin.

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TABLE II. Comparison of Clinical, Morphological and Biochemical Data in Three Non-Finnish Patients With Sialic Acid Storage Disease*

	Patient 1	Patient 2	Patient 3
Age at diagnosis/sex	2 months/M	11 months/F	16 years/F
Organomegaly	++	−	−
Coarse face	++	+	(+)
Skeletal abnormalities	−	−	−
Growth retardation	−	+	−
Developmental delay	+	+	+
Impaired speech	n/a	+	+
Ataxia	n/a	+	+
Vacuolated tissues	++	++ ++	
Free sialic acid in urine	++	++	++

*n/a = not applicable.

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